AMENDMENTS TO THE SPECIFICATION:

Please amend the specification as follows:

Delete pages 89-120, which is the paper copy of the Sequence Listing originally filed, and renumber pages 121-134 as pages 89-102 accordingly.

After the Figures, insert the Abstract.

Please insert the paper copy of the Sequence Listing submitted herewith into the specification after the Abstract.

Due to the fact that certain of the following paragraphs being amended below contain underlined text as originally filed, the added text is shown as double underlined text, and the deleted text is shown as ~~strike-through text~~.

Please amend the paragraph on page 5, line 9 which starts with "In another" as follows:

In another embodiment, Therapeutics which promote Notch function (hereinafter "Agonist Therapeutics") are administered for therapeutic effect; disorders which can thus be treated can be identified by *in vitro* assays such as described in Section 5.1, *infra*. Such Agonist Therapeutics include but are not limited to Notch proteins and derivatives thereof comprising the intracellular domain, and proteins that interact with Notch (*e.g.*, a protein comprising a Delta sequence homologous to *Drosophila* Delta amino acids 1-230 (see Figures 1A-1F ~~Figure 1~~ and SEQ ID NO:2), or comprising a Serrate sequence homologous to *Drosophila* Serrate amino acids 79-282 (see Figures 5A-5B ~~Figure 5~~ and SEQ ID NO:4)).

Please amend the paragraph on page 6, line 21 which starts with "Figure 1" as follows:

Figures 1A-1F ~~Figure 1~~. Primary Nucleotide Sequence of the Delta cDNA Dl1 (SEQ ID NO:1) and Delta amino acid sequence (SEQ ID NO:2). The DNA sequence of the 5′-3′ strand of the Dl1 cDNA is shown, which contains a number of corrections in comparison to that presented in Kopczynkski et al. (1988, Genes Dev. 2:1723-1735).

Please amend the paragraph on page 6, line 26 which starts with "Figure 2" as follows:

Figures 2A-2B ~~Figure 2~~. Notch Expression Constructs and the Deletion Mapping of the Delta/Serrate Binding Domain. S2 cells in log phase growth were transiently transfected with the series of expression constructs shown; the drawings represent the predicted protein products of the various Notch deletion mutants created. All expression constructs were derived from construct #1 pMtNMg. Transiently transfected cells were mixed with Delta expressing cells from the stably transformed line L49-6-7 or with transiently transfected Serrate expressing cells,

induced with $CuSO_4$, incubated under aggregation conditions and then scored for their ability to aggregate using specific antisera and immunofluorescence microscopy. Aggregates were defined as clusters of four or more cells containing both Notch and Delta/Serrate expressing cells. The values given for % Aggregation refer to the percentage of all Notch expressing cells found in such clusters either with Delta (Dl) (left column) or with Serrate (Ser) (right column). The various Notch deletion constructs are represented diagrammatically with splice lines indicating the ligation junctions. Each EGF repeat is denoted as a stippled rectangular box and numbers of the EGF repeats on either side of a ligation junction are noted. At the ligation junctions, partial EGF repeats produced by the various deletions are denoted by open boxes and closed brackets (for example see #23 ΔCla+EGF(10-12)). Constructs #3-13 represent the ClaI deletion series. As diagrammed, four of the ClaI sites, in repeats 7, 9, 17 and 26, break the repeat in the middle, immediately after the third cysteine (denoted by open box repeats; see Figure 3 for further clarification), while the fifth and most 3′ site breaks neatly between EGF repeats 30 and 31 (denoted by closed box repeat 31; again see Figure 3). In construct #15 split, EGF repeat 14 which carries the split point mutation, is drawn as a striped box. In construct #33 ΔCla+XEGF(10-13), the *Xenopus* Notch derived EGF repeats are distinguished from *Drosophila* repeats by a different pattern of shading. SP, signal peptide; EGF, epidermal growth factor repeat; N, Notch/lin-12 repeat; TM, transmembrane domain; cdc10, cdc10/ankyrin repeats; PA, putative nucleotide binding consensus sequence; opa, polyglutamine stretch termed opa; Dl, Delta; Ser, Serrate.

Please amend the paragraph on page 8, line 15 which starts with "Figure 5" as follows:

Figures 5A-5B ~~Figure 5~~. Nucleic Acid Sequence Homologies Between Serrate and Delta. A portion of the *Drosophila* Serrate nucleotide sequence (SEQ ID NO:3), with the encoded Serrate protein sequence (SEQ ID NO:4) written below (Fleming et al., 1990, Genes & Dev. 4:2188-2201 at 2193-94) is shown. The four regions showing high sequence homology with the *Drosophila* Delta sequence are numbered above the line and indicated by brackets. The total region of homology spans nucleotide numbers 627 through 1290 of the Serrate nucleotide sequence (numbering as in Figure 4 of Fleming et al., 1990, Genes & Dev. 4:2188-2201).

Please amend the paragraph on page 9, line 3 which starts with "Figure 8" as follows:

Figures 8A-8C ~~Figure 8~~. Nucleotide Sequences of Human Notch Contained in Plasmid cDNA Clone hN2k. Figure 8A: The DNA sequence (SEQ ID NO:5) of a portion of the human Notch insert is shown, starting at the EcoRI site at the 3′ end, and proceeding in the 3′ to 5′ direction. Figure 8B: The DNA sequence (SEQ ID NO:6) of a portion of the human Notch

insert is shown, starting at the EcoRI site at the 5′ end, and proceeding in the 5′ to 3′ direction. Figure 8C: The DNA sequence (SEQ ID NO:7) of a portion of the human Notch insert is shown, starting 3′ of the sequence shown in Figure 8B, and proceeding in the 5′ to 3′ direction. The sequences shown are tentative, subject to confirmation by determination of overlapping sequences.

Please amend the paragraph on page 9, line 13 which starts with "Figure 9" as follows:

Figures 9A-9B ~~Figure 9~~. Nucleotide Sequences of Human Notch Contained in Plasmid cDNA clone hN4k. Figure 9A: The DNA sequence (SEQ ID NO:8) of a portion of the human Notch insert is shown, starting at the EcoRI site at the 5′ end, and proceeding in the 5′ to 3′ direction. Figure 9B: The DNA sequence (SEQ ID NO:9) of a portion of the human Notch insert is shown, starting near the 3′ end, and proceeding in the 3′ to 5′ direction. The sequences shown are tentative, subject to confirmation by determination of overlapping sequences.

Please amend the paragraph on page 9, line 20 which starts with "Figure 10" as follows:

Figures 10A-10Q ~~Figure 10~~. DNA (SEQ ID NO:10) and Amino Acid (SEQ ID NO:11) Sequences of Human Notch Contained in Plasmid cDNA Clone hN3k.

Please amend the paragraph on page 9, line 22 which starts with "Figure 11" as follows:

Figures 11A-11G ~~Figure 11~~. DNA (SEQ ID NO:12) and Amino Acid (SEQ ID NO:13) Sequences of Human Notch Contained in Plasmid cDNA Clone hN5k.

Please amend the paragraph on page 9, line 24 which starts with "Figure 12" as follows:

Figures 12A-12C ~~Figure 12~~. Comparison of hN5k With Other Notch Homologs. Figure 12A. Schematic representation of *Drosophila* Notch. Indicated are the signal sequence (signal), the 36 EGF-like repeats, the three Notch/lin-12 repeats, the transmembrane domain (TM), the six CDC10 repeats, the OPA repeat, and the PEST (proline, glutamic acid, serine, threonine)-rich region. Figures 12B-12C ~~Figure 12B~~. Alignment of the deduced amino acid sequence of hN5k with sequences of other Notch homologs. Amino acids are numbered on the left side. The cdc10 and PEST-rich regions are both boxed, and individual cdc10 repeats are marked. Amino acids which are identical in three or more sequences are highlighted. The primers used to clone hN5k are indicated below the sequences from which they were designed. The nuclear localization sequence (NLS), casein kinase II (CKII), and cdc2 kinase (cdc2) sites of the putative CcN motif of the vertebrate Notch homologs are boxed. The possible bipartite nuclear targeting sequence (BNTS) and proximal phosphorylation sites of *Drosophila* Notch are also boxed.

Please amend the paragraph on page 10, line 7 which starts with "Figure 13" as follows:

Figures 13A-13H ~~Figure 13~~. Aligned amino acid sequences of Notch proteins of various species. humN: the human Notch protein encoded by the hN homolog (contained in part in plasmid hN5k) (SEQ ID NO:19). TAN-1: the human Notch protein encoded by the TAN-1 homolog (SEQ ID NO:20) (the sequence shown is derived partly from our own work and partly from the TAN-1 sequence as published by Ellisen et al., 1991, Cell 66:649-661); Xen N: Xenopus Notch protein (Coffman et al., 1990, Science 249:1438-1441). Dros N: Drosophila Notch protein (Wharton et al., 1985, Cell 43:567-581). Structural domains are indicated.

Please amend the paragraph on page 10, line 21 which starts with "Figure 15" as follows:

Figures 15A-15B ~~Figure 15~~. Immunocytochemical staining of colon tissue from a human patient with colon cancer. A colon tissue sample obtained from a patient with colon cancer was embedded in a paraffin section, and subjected to immunocytochemical staining with anti-human Notch monoclonal antibody P1, directed against the hN-encoded protein. Areas of increased staining are those areas in which malignant cells are present, as determined by cell morphology.

Please amend the paragraph on page 10, line 27 which starts with "Figure 16" as follows:

Figures 16A-16B ~~Figure 16~~. Immunocytochemical staining of cervical tissue. Human tissue samples were obtained, containing cancer of the cervix (Fig. 16A) or normal cervical epithelium (Fig. 16B) from the same patient, embedded in a paraffin section, and subjected to immunocytochemical staining with anti-human Notch monoclonal antibody directed against the TAN-1 protein. Areas containing malignant cells (as determined by morphology) exhibited increasing staining relative to non-malignant cells. Among non-malignant cells, connective tissue and the basal layer of the epithelium (containing stem cells) stained with the anti-Notch antibody.

Please amend the paragraph on page 11, line 5 which starts with "Figure 17" as follows:

Figures 17A-17L ~~Figure 17~~. DNA (SEQ ID NO:21) and encoded amino acid sequence (contained in SEQ ID NO:19) of human Notch homolog hN. The entire DNA coding sequence is presented (as well as noncoding sequence), with the exclusion of that encoding the initiator Met.

Please amend the paragraph on page 12, line 8 which starts with "In another" as follows:

In another embodiment, Therapeutics which promote Notch function (hereinafter "Agonist Therapeutics") are administered for therapeutic effect; disorders which can thus be treated can be identified by *in vitro* assays such as described in Section 5.1, *infra*. Such Agonist

Therapeutics include but are not limited to Notch proteins and derivatives thereof comprising the intracellular domain, Notch nucleic acids encoding the foregoing, and proteins comprising toporythmic protein domains that interact with Notch (*e.g.*, a protein comprising an extracellular domain of a Delta protein or a Delta sequence homologous to *Drosophila* Delta amino acids 1-230 (see Figures 1A-1F ~~Figure 1~~ and SEQ ID NO:2), or comprising a Serrate sequence homologous to *Drosophila* Serrate amino acids 79-282 (see Figures 5A-5B ~~Figure 5~~ and SEQ ID NO:4)).

Please amend the paragraph on page 38, line 23 which starts with "In other" as follows:

In other specific embodiments, the invention provides nucleotide sequences and subsequences of Notch, preferably human Notch, consisting of at least 25 nucleotides, at least 50 nucleotides, or at least 150 nucleotides. Nucleic acids encoding the proteins and protein fragments described above are provided, as well as nucleic acids complementary to and capable of hybridizing to such nucleic acids. In one embodiment, such a complementary sequence may be complementary to a Notch cDNA sequence of at least 25 nucleotides, or of at least 100 nucleotides. In a preferred aspect, the invention utilizes cDNA sequences encoding human Notch or a portion thereof. In a specific embodiment, such sequences of the human Notch gene or cDNA are as contained in plasmids hN3k, hN4k, or hN5k (see Section 9, *infra*) or in the gene corresponding thereto; such a human Notch protein sequence can be as shown in Figures 10A-10Q ~~Figures 10~~ (SEQ ID NO:11) or Figures 11A-11G [[~~11~~]] (SEQ ID NO:13). In other embodiments, the Notch nucleic acid and/or its encoded protein has at least a portion of the sequence shown in one of the following publications: Wharton et al., 1985, Cell 43:567-581 (*Drosophila* Notch); Kidd et al., 1986, Mol. Cell. Biol. 6:3094-3108 (*Drosophila* Notch); Coffman et al., 1990, Science 249:1438-1441 (*Xenopus* Notch); Ellisen et al., 1991, Cell 66:649-661 (a human Notch). In another aspect, the sequences of human Notch are those encoding the human Notch amino acid sequences or a portion thereof as shown in Figures 13A-13H ~~Figure 13~~. In a particular aspect, the human Notch sequences are those of the hN homolog (represented in part by plasmid hN5k) or the TAN-1 homolog.

Please amend the paragraph on page 39, line 14 which starts with "In one" as follows:

In one embodiment of the invention, the invention is directed to the full-length human Notch protein encoded by the hN homolog as depicted in Figures 13A-13H ~~Figure 13~~, both containing the signal sequence (*i.e.*, the precursor protein; amino acids 1-2169) and lacking the signal sequence (*i.e.*, the mature protein; amino acids ~26-2169), as well as portions of the foregoing (*e.g.*, the extracellular domain, EGF homologous repeat region, EGF-like repeats 11

and 12, cdc-10/ankyrin repeats, etc.) and proteins comprising the foregoing, as well as nucleic acids encoding the foregoing.

Please amend the paragraph on page 43, line 8 which starts with "Similar methods" as follows:

Similar methods to those described *supra* can be used to obtain a nucleic acid encoding Delta, Serrate, or adhesive portions thereof, or other toporythmic gene of interest. In a specific embodiment, the Delta nucleic acid has at least a portion of the sequence shown in Figures 1A-1F ~~Figure 1~~ (SEQ ID NO:1). In another specific embodiment, the Serrate nucleic acid has at least a portion of the sequence shown in Figures 5A-5B ~~Figure 5~~ (SEQ ID NO:3). The nucleic acid sequences encoding toporythmic proteins can be isolated from porcine, bovine, feline, avian, equine, or canine, as well as primate sources and any other species in which homologs of known toporythmic genes [including but not limited to the following genes (with the publication of sequences in parentheses): Delta (Vassin et al., 1987, EMBO J. 6, 3431-3440; Kopczynski et al., 1988, Genes Dev. 2, 1723-1735; note corrections to the Kopczynski et al. sequence found in Figure 1 hereof (SEQ ID NO:1 and SEQ ID NO:2)) and Serrate (Fleming et al., 1990, Genes & Dev. 4, 2188-2201)] can be identified. Such sequences can be altered by substitutions, additions or deletions that provide for functionally equivalent molecules, as described *supra*.

Please amend the paragraph on page 53, line 20 which starts with "In a specific" as follows:

In a specific embodiment, the adhesive fragment of Notch is that comprising the portion of Notch most homologous to ELR 11 and 12, i.e., amino acid numbers 447 through 527 (SEQ ID NO:14) of the *Drosophila* Notch sequence (see Figure 4). In yet another specific embodiment, the adhesive fragment of Delta mediating binding to Notch is that comprising the portion of Delta most homologous to about amino acid numbers 1-230 of the *Drosophila* Delta sequence (SEQ ID NO:2). In a specific embodiment relating to an adhesive fragment of Serrate, such fragment is that comprising the portion of Serrate most homologous to about amino acid numbers 85-283 or 79-282 of the *Drosophila* Serrate sequence (see Figures 5A-5B ~~Figure 5~~ (SEQ ID NO:4)).

Please amend the paragraph on page 56, line 17 which starts with "Various" as follows:

Various procedures known in the art may be used for the production of polyclonal antibodies to a Notch protein or peptide. In a particular embodiment, rabbit polyclonal antibodies to an epitope of the human Notch protein encoded by a sequence depicted in Figures

10A-10Q or Figures 11A-11G ~~Figure 10 or 11~~, or a subsequence thereof, can be obtained. For the production of antibody, various host animals can be immunized by injection with the native Notch protein, or a synthetic version, or fragment thereof, including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to increase the immunological response, depending on the host species, and including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhold limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum.

Please amend the paragraph on page 58, line 28 which starts with "For the" as follows:

For the Delta expression construct, the Dl1 cDNA (Kopczynski et al., 1988, Genes Dev. 2, 1723-1735; Figures 1A-1F ~~Figure 1~~; SEQ ID NO:1), which includes the complete coding capacity for Delta, was inserted into the EcoRI site of pRmHa-3. This construct was called pMTDl1.

Please amend the paragraph on page 69, line 1 which starts with "Schematic" as follows:

Schematic drawings of the constructs tested and results of the aggregation experiments are shown in Figures 2A-2B ~~Figure 2~~. To assay the degree of aggregation, cells were stained with antisera specific to each gene product and examined with immunofluorescent microscopy. Aggregates were defined as clusters of four or more cells containing both Notch and Delta expressing cells, and the values shown in Figures 2A-2B ~~Figure 2~~ represent the percentage of all Notch expressing cells found in such clusters. All numbers reflect the average result from at least two separate transfection experiments in which at least 100 Notch expressing cell units (either single cells or clusters) were scored.

Please amend the paragraph on page 69 line 24 which starts with "The initial" as follows:

The initial constructs (#2 DSph and #3 ΔCla) deleted large portions of the EGF repeats. Their inability to promote Notch-Delta aggregation suggested that the EGF repeats of Notch were involved in the interaction with Delta. A series of six in-frame ClaI restriction sites was used to further dissect the region between EGF repeats 7 and 30. Due to sequence homology between repeats, five of the ClaI sites occur in the same relative place within the EGF repeat, just after the third cysteine, while the sixth site occurs just before the first cysteine of EGF repeat 31 (Figure 3). Thus, by performing a partial ClaI digestion and then religating, deletions were obtained that not only preserved the open reading frame of the Notch protein but in

addition frequently maintained the structural integrity and conserved spacing, at least theoretically, of the three disulfide bonds in the chimeric EGF repeats produced by the religation (Figures 2A-2B ~~Figure 2~~, constructs #4-14). Unfortunately, the most 3′ ClaI site was resistant to digestion while the next most 3′ ClaI site broke between EGF repeats 30 and 31. Therefore, when various ClaI digestion fragments were reinserted into the framework of the complete ClaI digest (construct #3 ΔCla), the overall structure of the EGF repeats was apparently interrupted at the 3′ junction.

Please amend the paragraph on page 73 line 14 which starts with "PCR primers" as follows:

PCR primers based on the *Xenopus* Notch sequence (Coffman et al., 1990, Science 249, 1438-1441) were used to obtain an ~350 bp fragment from a *Xenopus* Stage 17 cDNA library that includes EGF repeats 11 and 12 flanked by half of repeats 10 and 13 on either side. This fragment was cloned into construct #3 ΔCla, and three independent clones were tested for ability to interact with Delta in the cell culture aggregation assay. Two of the clones, #33a&bΔCla+XEGF(10-13), when transfected into S2 cells were able to mediate Notch-Delta interactions at a level roughly equivalent to the analogous *Drosophila* Notch construct #19ΔCla+EGF(10-13), and again in a calcium dependent manner (Table III). However, the third clone #33cΔCla+XEGF(10-13) failed to mediate Notch-Delta interactions although the protein was expressed normally at the cell surface as judged by staining live unpermeabilized cells. Sequence comparison of the *Xenopus* PCR product in constructs #33a and 33c revealed a missense mutation resulting in a leucine to proline change (amino acid #453, Coffman, et al., 1990, Science 249, 1438-1441) in EGF repeat 11 of construct #33c. Although this residue is not conserved between *Drosophila* and *Xenopus* Notch (Figures 8A-8C ~~Figure 8~~), the introduction of a proline residue might easily disrupt the structure of the EGF repeat, and thus prevent it from interacting properly with Delta.

Please amend the paragraph on page 75 line 3 which starts with "The finding" as follows:

The finding that EGF repeats 11 and 12 of Notch form a discrete Delta binding unit represents the first concrete evidence supporting the idea that each EGF repeat or small subset of repeats may play a unique role during development, possibly through direct interactions with other proteins. The homologies seen between the adhesive domain of Delta and Serrate (Figures 5A-5B ~~Figure 5~~) suggest that the homologous portion of Serrate is "adhesive" in that it mediates

binding to other toporythmic proteins (see Section 8, *infra*). In addition, the gene scabrous, which encodes a secreted protein with similarity to fibrinogen, may interact with Notch.

Please amend the paragraph on page 76 line 17 which starts with "Serrate" as follows:

Serrate expressing cells adhered to Notch expressing cells in a calcium dependent manner (Figures 2A-2B ~~Figure 2~~ and Rebay et al., 1991, *supra*). However, unlike Delta, under the experimental conditions tested, Serrate did not appear to interact homotypically. In addition, no interactions were detected between Serrate and Delta.

Please amend the paragraph on page 76 line 22 which starts with "A subset" as follows:

A subset of Notch deletion constructs were tested, and showed that EGF repeats 11 and 12, in addition to binding to Delta, also mediate interactions with Serrate (Figures 2A-2B ~~Figure 2~~; Constructs #1, 7-10, 13, 16, 17, 19, 28, and 32). In addition, the Serrate-binding function of these repeats also appears to have been conserved in the corresponding two EGF repeats of Xenopus Notch (#33ΔCla+XEGF(10-13)). These results unambiguously show that Notch interacts with both Delta and Serrate, and that the same two EGF repeats of Notch mediate both interactions. The Serrate region which is essential for the Notch/Serrate aggregation was also defined. Deleting nucleotides 676-1287 (i.e. amino acids 79-282) (See Figures 5A-5B ~~Figure 5~~; SEQ ID NO:3 and NO:4) eliminates the ability of the Serrate protein to aggregate with Notch.

Please amend the paragraph on page 78 line 1 which starts with "The sequence" as follows:

The sequence of various portions of Notch contained in the cDNA clones was determined (by use of Sequenase®, U.S. Biochemical Corp.) and is shown for hN2k and hN4k in Figures 8A-8C ~~Figures 8~~ (SEQ ID NO:5-7) and Figures 9A-9B [[9]] (SEQ ID NO:8, 9), respectively. Further sequence analysis of hN2k revealed that it encodes a human Notch sequence overlapping that contained in hN5k.

Please amend the paragraph on page 78 line 6 which starts with "The complete" as follows:

The complete nucleotide sequences of the human Notch cDNA contained in hN3k and hN5k was determined by the dideoxy chain termination method using the Sequenase® kit (U.S. Biochemical Corp.). Those nucleotide sequences encoding human Notch, in the appropriate reading frame, were readily identified since there are no introns and translation in only one out of the three possible reading frames yields a sequence which, upon comparison with the published *Drosophila* Notch deduced amino acid sequence, yields a sequence with a substantial

degree of homology to the *Drosophila* Notch sequence. The DNA and deduced protein sequences of the human Notch cDNA in hN3k and hN5k are presented in Figures 10A-10Q ~~Figures 10~~ (SEQ ID NO:10, 11) and Figures 11A-11G [[~~11~~]] (SEQ ID NO:12, 13), respectively. Clone hN3k encodes a portion of a Notch polypeptide starting at approximately the third Notch/lin-12 repeat to several amino acids short of the carboxy-terminal amino acid. Clone hN5k encodes a portion of a Notch polypeptide starting approximately before the cdc10 region through the end of the polypeptide, and also contains a 3′ untranslated region.

Please amend the paragraph on page 78 line 21 which starts with "Comparing the" as follows:

Comparing the DNA and protein sequences presented in Figures 10A-10Q ~~Figure 10~~ (SEQ ID NO:10, 11) with those in Figures 11A-11G ~~Figure 11~~ (SEQ ID NO:12, 13) reveals significant differences between the sequences, suggesting that hN3k and hN5k represent part of two distinct Notch-homologous genes. The data thus suggest that the human genome harbors more than one Notch-homologous gene. This is unlike *Drosophila*, where Notch appears to be a single-copy gene.

Please amend the paragraph on page 79 line 3 which starts with "The amino" as follows:

The amino acid sequence shown in Figures 10A-10Q ~~Figure 10~~ (hN3k) was compared with the predicted sequence of the TAN-1 polypeptide shown in Figure 2 of Ellisen et al., August 1991, Cell 66:649-661. Some differences were found between the deduced amino acid sequences; however, overall the hN3k Notch polypeptide sequence is 99% identical to the corresponding TAN-1 region (TAN-1 amino acids 1455 to 2506). Four differences were noted: in the region between the third Notch/lin-12 repeat and the first cdc10 motif, there is an arginine (hN3k) instead of an X (TAN-1 amino acid 1763); (2) there is a proline (hN3k) instead of an X (TAN-1, amino acid 1787); (3) there is a conservative change of an aspartic acid residue (hN3k) instead of a glutamic acid residue (TAN-1, amino acid 2495); and (4) the carboxyl-terminal region differs substantially between TAN-1 amino acids 2507 and 2535.

Please amend the paragraph on page 79 line 15 which starts with "The amino" as follows:

The amino acid sequence shown in Figures 11A-11G ~~Figure 11~~ (hN5k) was compared with the predicted sequence of the TAN-1 polypeptide shown in Figure 2 of Ellisen et al., August 1991, Cell 66:649-661. Differences were found between the deduced amino acid sequences. The deduced Notch polypeptide of hN5k is 79% identical to the TAN-1 polypeptide

(64% identical to *Drosophila* Notch) in the cdc10 region that encompasses both the cc10 motif (TAN-1 amino acids 1860 to 2217) and the well-conserved flanking regions (Figs. 12A-12C ~~Fig. 12~~). The cdc10 region covers amino acids 1860 through 2217 of the TAN-1 sequence. In addition, the hN5k encoded polypeptide is 65% identical to the TAN-1 polypeptide (44% identical to *Drosophila* Notch) at the carboxy-terminal end of the molecule containing a PEST (proline, glutamic acid, serine, threonine)-rich region (TAN-1 amino acids 2482 to 2551) (Figs. 12B-12C ~~Fig. 12B~~). The stretch of 215 amino acids lying between the aforementioned regions is not well conserved among any of the Notch-homologous clones represented by hN3k, hN5k, and TAN-1. Neither the hN5k polypeptide nor *Drosophila* Notch shows significant levels of amino acid identity to the other proteins in this region (*e.g.*, hN5k/TAN-1 = 24% identity; hN5k/*Drosophila* Notch = 11% identity; TAN-1/*Drosophila* Notch = 17% identity). In contrast, *Xenopus* Notch (Xotch) (SEQ ID NO:16), rat Notch (SEQ ID NO:17), and TAN-1 (SEQ ID NO:18) continue to share significant levels of sequence identity with one another (*e.g.*, TAN-1/rat Notch = 75% identity, TAN-1/*Xenopus* Notch = 45% identity, rat Notch/*Xenopus* Notch = 50% identity).

Please amend the paragraph on page 80 line 6 which starts with "Examination" as follows:

Examination of the sequence of the intracellular domains of the vertebrate Notch homologs shown in Figures 12B-12C ~~Figure 12B~~ revealed an unexpected finding: all of these proteins, including hN5k, contain a putative CcN motif, associated with nuclear targeting function, in the conserved region following the last of the six cdc10 repeats (Figs. 12B-12C ~~Fig. 12B~~). Although *Drosophila* Notch lacks such a defined motif, closer inspection of its sequence revealed the presence of a possible bipartite nuclear localization sequence (Robbins et al., 1991, Cell 64:615-623), as well as of possible CK II and cdc2 phosphorylation sites, all in relative proximity to one another, thus possibly defining an alternative type of CcN motif (Figs. 12B-12C ~~Fig. 12B~~).

Please amend the paragraph on page 81 line 1 which starts with "The 5′ sequence" as follows:

The 5′ sequence of our isolated TAN-1 homolog has been determined through nucleotide number 972 (nucleotide number 1 being the A in the ATG initiation codon), and compared to the sequence as published by Ellisen et al (1991, Cell 66:649-661). At nucleotide 559, our TAN-1 homolog has a G, whereas Ellisen et al. disclose an A, which change results in a different encoded amino acid. Thus, within the first 324 amino acids, our TAN-1-encoded

protein differs from that taught by Ellisen et al., since our protein has a Gly at position 187, whereas Ellisen et al. disclose an Arg at that position (as presented in Figures 13A-13H). ~~Figure 13.)~~

Please amend the paragraph on page 81 line 10 which starts with "The full-length" as follows:

The full-length amino acid sequences of both the hN (SEQ ID NO:19) and TAN-1-encoded (SEQ ID NO:20) proteins, as well as Xenopus and Drosophila Notch proteins, are shown in Figures 13A-13H ~~Figure 13~~. The full-length DNA coding sequence (except for that encoding the initiator Met) (contained in SEQ ID NO:21) and encoded amino acid sequence (except that the initiator Met is not shown) (contained in SEQ ID NO:19) of hN are shown in Figures 17A-17L ~~Figure 17~~.

Please amend the paragraph on page 86 line 11 which starts with "In all tumors" as follows:

In all tumors examined, the Notch proteins encoded by both human Notch homologs TAN-1 and hN were present at increased levels in the malignant part of the tissue compared to the normal part. Representative stainings are shown in the pictures provided (Figs. 14-16B) ~~(Figs. 14-16)~~.